UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

003578

MEMORANDUM

TO: Robert Taylor, PM 25
Registration Division (TS-767)

12-16-83

THRU: Robert R. Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769)

SUBJECT: 476-EUP-RNE and 476-EUP-RNG for SC-0224 4-LC Nonselective Foliar Systemic Herbicide for Weed Control in Non Food Crop Areas. Trimethylsulfonium carboxymethylaminomethylphosphonate. R-50224. CASWELL [illegible]
Accession Nos. 249801, 249802, 249803, 249804.

Applicant: Stauffer Chemical Company
1200 S. 47th Street
Richmond, California 94804

Stauffer Chemical Company requests an experimental use permit for SC-0224 4-LC non-selective foliar systemic herbicide for weed control in non food areas. The use will include railroad, highway, and other rights of way use patterns in the states of Colorado, Iowa, Kansas, Minnesota, Nebraska, North and South Dakota, Missouri, Illinois, Indiana, Michigan, Wisconsin, Conn., Delaware, Kentucky, Maine, New Jersey, New York, Ohio, Pennsylvania, Virginia, Florida, Georgia, North and South Carolina, Tennessee, Louisiana, Mississippi, New Mexico, Oklahoma, Texas, Arizona, Nevada, Utah, Idaho, Montana, Oregon, Washington, and Wyoming; 41 of the 50 states. The data are needed to support future registrations of SC-0224 4-LC. The proposed experimental program is from Sept. 1, 1983 to September 1, 1985.

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Reccomendations:

1. The experimental use permit of the herbicide SC-0224 4-LC is supported by available toxicity data.

2. The registrant should be apprised of the deficiencies reported for the following mutagenicity studies:

   A. Bone Marrow Cytogenetic Analysis in Rats

   B. Sex-Linked Recessive Lethal Test in Drosophila melanogaster.

   C. Mutagenicity evaluation in Salmonella typhimurium (Lot #7269-10) and (Lot #7646-0901).

   D. Chinese Hamster Ovary Cytogenetic Assay (Lot #6841-48-3 and Lot #7466-18-01).

   E. Mouse Lymphoma Multiple Endpoint Test Forward Mutation Assay (Lot #7269-10 and 6841-48-3).

   F. Morphological Transformation of Balb/3T3 Cells (Lot #7269-10).

Reviews of individual studies are attached. The mutagenicity studies were reviewed jointly by R. A. Gessert and John Chen except for the Drosophila study which was reviewed by John Chen. All Other toxicity studies were reviewed by R. A. Gessert.

3. Acute Oral Toxicity for 62% technical chemical in Sprague Dawley Rats:

   Male = 748 mg/kg
   Female = 755 mg/kg
   Toxicity Category III. Core Minimum data.

4. Acute Dermal Toxicity of 62% Technical Chemical in Stauffland Albino Rabbits:

   Intact and Abraded Skin; Male and Female: LD_{50} > 2000 mg/kg.
   Toxicity Category III
   Core Supplementary Study in the absence of complete individual animal data.

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5. Primary Skin Irritation of 62% technical chemical in Stauffland Albino Rabbits:

Draize Primary Irritation Score = 0.67 - a mild dermal irritant.
Toxicity Category IV.
Core Minimum data.

- Primary Eye Irritation of 62% technical chemical in Stauffland Albino Rabbits:

a. No corneal involvement.

b. Mild irritation to conjunctivae. Irritation is reduced by washing eyes.

c. Toxicity Category III.

d. Data are Core Minimum.

- Acute Inhalation Toxicity of 62% technical chemical in Sprague Dawley Rats:

a. LC_{50} > 6.9 mg/liter (Male/Female).

b. Data are Core Minimum.

- Teratology in CD Rats:

a. Maternal toxicity (reduced body weights and feed intake) was demonstrated in high dose group (333 mg/kg).

b. Fetotoxicity seen as reduced fetal body weight in high dose groups, apparently resulting from maternal toxicity.

c. Teratogenicity was not demonstrated.

d. Data are considered supplementary, pending submission of additional information requested to clarify the actual dose administered in terms of mg/kg b.w.

Acute Oral Toxicity of SC-0224-4LC Formulation in Rats:

a. Male - LD_{50} = 846 mg/kg.

b. Female - LD_{50} = 805 mg/kg.

c. Toxicity Category III.

d. Core Minimum Data.

10. Acute Dermal Toxicity of SC-0224-4-LC Formulation in Rabbits:

    a. LD_{50}, intact and abraded skin = 1279 mg/kg.

    b. Toxicity Category II.

    c. Core Minimum Data

11. Primary Dermal Irritation of SC-0224-4-LC Formulation in Rabbits:

    a. Formulation is a Moderate Dermal Irritant

    b. Toxicity Category III.

    c. Core Minimum Data.

12. Primary Eye Irritation of SC-0224-4-LC Formulation in Rabbits:

    a. Formulation is very corrosive to the eye, producing corneal opacity, iritis, and severe conjunctivitis. This contrasts with the technical chemical, which is only mildly irritating to the eye.

    b. The irritation and corrosiveness is reduced somewhat by washing the eyes.

    c. Toxicity Category I.

    d. The data are Core Minimum.

13. The proposed labels for both the "concentrate" and for the 4 LC formulation are satisfactory, and accurately reflects the corrosive nature of the formulation to the eyes, in the case of the formulation.

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Veterinary Medical Officer
Toxicology Review Section I

TS-769:GESSERT:s11:X73710:9/19/83 card 7
11/7/83

Acute Oral Toxicity of SC-0224 Technical Chemical in Male and Female Sprague-Dawley Rats. Conducted by T. Billow and D. Crume; Stauffer Chemical Co.; Richmond Toxicology Laboratory; Richmond, California. Report T-11185. October-November, 1982. Accession No. 249802. Caswell No. 893C.

Material Tested:

Trimethylsulfonium carboxymethylaminoethyl-phosphonate. SC-0224 Technical Chemical. Lot #7981-12-01. An herbicide of 62% purity.

Male Rats: After 16-18 hours fasting ten male rats per dose level were treated by oral gavage at the rate of 500, 550, 700, 800, or 900 mg/kg. Twenty rats were dosed with 600 mg/kg. Mortality was 10/10, 6/10, 3/10, and 5/20 for doses of 900 mg/kg, 800 mg/kg, 700 mg/kg, and 600 mg/kg, respectively. The oral LD_{50} for male rats was 748 mg/kg, with 95% confidence limits of 667-840 mg/kg.

Female Rats: After 16-18 hours fasting ten female rats per dose level were treated by oral gavage at the rate of 550, 700, 800, and 900 mg technical chemical per kg body weight. Mortality was 1/10, 5/10, 4/10, and 9/10 for doses of 550, 700, 800, and 900 mg/kg, respectively. The oral LD_{50} for female rats was 755 mg/kg with 95% confidence limits of 677-841 mg/kg.

Clinical Signs:

A single dose of 900 mg/kg killed 9 male rats within 4 hours and 9 female rats within 24 hours. Adverse clinical signs included mild to severe depression, prostration, ptosis, slow and shallow respiration, and tremors.

Necropsy findings in rats dying on test were dark red spotted lungs, distended bladder filled with dark fluid, darkened livers, and dark spleens. Survivors necropsied on day 14 appeared normal.

Controls: Forty males and 40 females dosed with water appeared normal throughout the 14-day observation period and on necropsy at day 14.

The data meet Core-Minimum standards.

Toxicity Category - III.

Acute Dermal Toxicity of SC-0224 Technical Chemical in Stauffland Albino Rabbits - Abraded Skin. Conducted by A. Howell, B. Jones, and S. Sorenson; Stauffer Chemical Co.; Richmond Toxicology Laboratory; Richmond, California. Report T-11185. October-November, 1982. Accession No. 249802. Caswell No. 893C.

Material Tested:

Trimethylsulfonium carboxymethylaminoethylphosphonate. SC-0224 Technical Chemical. Lot #79810-12-01. An herbicide of 62% purity.

Four male and female Stauffland albino rabbits per dose level had the test material applied to the clipped abraded abdominal skin under a protective binder. Dose levels were 2200, 1900, 1600, 1200, 1000, 900, and 800 mg/kg body weight. Three male and three female rabbits served as zero dose controls. After 24 hours the binder and the test material were removed, the treatment sites were inspected for irritation, and the abdomens were rewrapped in a gauze binder. Three days later the gauze binder was removed, and the rabbits were observed for 14 days following initial treatment. Necropsies were performed on all rabbits that died during the study and on all survivors at 14 days.

Results:

Three of 8 rabbits died at 2200 mg/kg; 2/8 at 1900 mg/kg; 3/8 at 1600 mg/kg; 2/8 at 1200 mg/kg; 1/8 at 1000 mg/kg; and 1/8 at 900 mg/kg. There was no mortality at 800 mg/kg or in the controls.

Necropsy Effects seen in rabbits which died on test included red and purple areas below the right kidney in one rabbit at 2200, 1900, and 1600 mg/kg and also pale lungs in 1 rabbit at each of these doses.

Rabbits which survived to 14 days all appeared normal on necropsy.

Clinical Signs were mild to severe depression in some rabbits at all treatment levels.

Local Dermal Effects was a mild to moderate erythema after 24 hour exposure.

Clinical signs and local dermal effects did not appear to be related to dose.

Acute Dermal Toxicity of SC-0224 Technical Chemical in Stauffland Albino Rabbits - Intact Skin.

This portion of the study was conducted in the same manner as for the abraded skin. A single dose level of 2000 mg/kg body weight was used. There was no mortality. The only clinical sign observed was mild depression, all rabbits appearing normal by day 2.

The only local dermal effect was mild erythema after 24 hour exposure.

Necropsy on day 14 revealed no abnormal findings.

However, "data" were provided in a summary form. Individual animal data and observations were not provided. Individual necropsy reports were not provided. The data therefore are classified Core supplementary. Upon submission of complete individual animal data the core classification possibly may be upgraded.

Conclusions:

1. Acute dermal LD_{50} in male and female rabbits (intact and abraded skin) was greater than 2000 mg/kg.

2. Toxicity Category III.

3. Data are Core Supplementary.

Primary Skin Irritation of SC-0224 Technical Chemical in Stauffland Albino Rabbits. Conducted by T. Billow and D. Crume. Stauffer Chemical Company. Richmond Toxicology Laboratory; Richmond California. Report T-11185. October-November, 1982. Accession No. 249,802. Caswell No. 893C.

Material Tested:

Trimethylsulfonium carboxymethylaminoethylphosphonate. SC-0224 Technical Chemical. Lot #7981-12-01. An herbicide of 62% purity. pH 5.65.

Twenty-four Hour Exposure:

Six Stauffland albino rabbits were used in the study. One-half ml of SC-0224 technical chemical was placed on an abraded site and an intact site and covered with a one-inch square gauze patch. This was secured by adhesive tape and wrapped with rubberized damming for 24 hours. After 24 hours the coverings and test material were removed and the reactions scored. The site reactions were also scored after 72 hours.

Results:

At 24 hours mild erythema was observed in all 6 rabbits in intact and abraded skin. Mild edema was seen in abraded sites in 3 rabbits and in the intact site in one additional rabbit. At 72 hours the irritation had subsided, with mild edema being seen in 1 intact site of only 1 rabbit. The Primary Irritation Score (Draize) was 0.67.

Four-Hour Exposure:

The above procedure was also conducted with an exposure time of 4 hours and observations and scoring being made after 4 hours, 24 hours, and 72 hours.

After 4 hours mild erythema was seen at the abraded sites in 4 rabbits. In 2 of these rabbits the erythema persisted for 24 hours. In one of the rabbits mild edema was also seen at 4 hours, but not subsequently. No reactions were seen at 72 hours. The Primary Irritant Score was 0.19.

Conclusions:

1. SC-0224 technical chemical is a mild dermal irritant.
2. Toxicity Category - IV.
3. Data are Core-Minimum.

Primary Eye Irritation of SC-0224 Technical Chemical in Stauffland Albino Rabbits. Conducted by T. Billow and D. Crume. Stauffer Chemical Company. Richmond Toxicology Laboratory; Richmond California. Report T-11185. October-November, 1982. Accession No. 249802. Caswell No. 893C.

Material Tested:

Trimethylsulfonium carboxymethylaminoethylphosphonate. SC-0224 Technical Chemical. Lot #7981-12-01. 62% purity. pH 5.65.

Nine rabbits were treated by placing 0.1 ml of the chemical inside the lower lid of one eye of each rabbit. In 3 of the rabbits the eye was washed with water 20-30 seconds after exposure, and in the remaining 6 rabbits the eye was not washed. Untreated eyes served as negative controls. The cornea, iris, and conjunctivae were observed at 24, 48, and 72 hours, and at 4, 7, 10, 11, and 14 days after treatment. Scoring was according to the method of Draize.

Results:

There was no corneal involvement in any of the eyes. In the unwashed eyes, mild iritis was seen in 1 rabbit and mild conjunctivitis in all 6 rabbits at 24 hours. All unwashed eyes were cleared by 7 days.

In the 3 washed eyes mild conjunctivitis was seen in 2 of the rabbits. This had cleared by day 3.

Conclusions:

1. SC-0224 is mildly irritating to the conjunctivae. The irritation is reduced by washing.

2. Toxicity Category - III.

3. The data are Core-Minimum.

Acute Inhalation Toxicity of SC-0224 Technical Chemical in Sprague-Dawley Rats. Conducted by Stephen MacAskill, M. R. Chaffee, et al., Stauffer Chemical Company, Environmental Health Center Inhalation Facility, 400 Farmington Ave., Farmington, Connecticut 06032. September 1982. Accession No. 249802. Study No. T-11084. Caswell #893C.

Material Tested:

Trimethylsulfonium carboxymethylaminoethylphosphonate. SC-0224 Technical Chemical. Lot #7981-12-01. 62% purity. pH 5.65.

Procedure:

Before exposing the rats to inhalation exposure of the test chemical, the equipment was adjusted and tested to produce the highest attainable concentration, about 5 mg/L (aerosol plus vapor). This was done by regulating chamber airflow, generator airflow, and generator "dilution airflow."

Ten male and 10 female Sprague-Dawley rats, 8-10 weeks old were then exposed to this regulator atmosphere. They were caged individually within the chamber, having first been weighed and ear-tagged.

The rats were exposed to the test atmosphere for 4 hours with a chamber flow of 17.4 air changes per hour, and then remained in the chamber for an additional hour with air only in order to air flush the chemical from their pelts.

Ten male and 10 female rats were then similarly treated in a clean chamber, but using air only instead of the test atmosphere.

The rats were observed during exposure and then twice daily for 14 days. They were weighed on the day of exposure, on days 2 and 7 after exposure and on day 14 at the end of the experiment. On day 14 the rats were anesthetized by sodium pentobarbital injection, killed by exsanguination, and necropsied. The following tissues were collected, fixed in 10% neutral buffered formalin and held for future histopathological examination, if needed: trachea, larynx, bronchi, nasal passages, lungs, liver, spleen, kidneys, adrenals, heart, and any tissues which appeared abnormal.

Results:

The nominal concentration of the chemical was 14.93 mg chemical per liter of atmosphere. (Weight of test substance used during exposure/total chamber air flow).

The acutal measured chamber concentration of the chemical was 6.92 mg per liter of atmosphere.

The Mass Medium Aerodynamic Diameter of the aerosolized chemical particles was 3.5 um (at 64 minutes) and 2.8 um (at 184 minutes).

The only effects seen during treatment were wet fur (4 rats) and salivation. Most of the rats exposed to the chemical also had stained fur for one day following exposure. 6 male rats and 2 female rats also showed chromorhinorrhea, especially on the day of exposure (day 0).

In body weight after 14 days, the females exposed to the chemical weighed 99% of their pre-exposure weight whereas the exposed males and all the controls showed a net weight gain of 5-12% over the 14 day observation period, the greater weight gains being seen in the controls.

On necropsy there were no findings which could be related to exposure.

Conclusions:

1. The $LC_{50} > 6.9$ mg/liter.

2. Data meet Core-Minimum standards.

A Teratology Study in CD Rats with SC-0224. Study T-11050. Conducted by Stauffer Chemical Co. Environmental Health Center, Farmington, Conn. 06032. Study conducted by J. R. Downs, J. L. Minor, G. M. Zwicker, et al. November 1982. Accession No. 249802. Caswell #893C.

Material Tested:

Trimethylsulfonium carboxymethylaminoethylphosphonate. SC-0224. Lot #EHC-0355-25. SC-0224. Purity (potency) 19.2% in [redacted].

Sprague-Dawley rats were obtained from Charles River Breeding Laboratories in Kingston, N.Y. Thirty males and 145 females were used for mating. One or 2 females were placed with one male. The next morning the females were examined for the presence of a copulatory plug or sperm in a vaginal smear. The day sperm or a plug were found was considered day 0 of gestation for that female. About 25 mated females were assigned to each of 4 dose groups and were intubated with 0, 30, 100, or 333 mg/kg 0224 active chemical in water on day 6 through 20 of gestation. Body weights and feed consumption were recorded on days 0, 6, 9, 12, 16 and 21. The females were killed on day 21 of gestation and necropsied. The reproductive tract was weighed and examined, and resorption sites were noted. Corpora lutea were counted on the ovaries. Fetuses were weighed, sexed, and examined for external malformations. One-half of the fetuses were eviscerated, fixed in alcohol, and then cleared and stained with 1% KOH and alizarin red S for skeletal anomaly examination. The heads of the rest of the fetuses were removed and fixed in Bouin's fixative; the trunks of these fetuses underwent internal organ examination under a dissecting microscope prior to being eviscerated and processed for skeletal examination.

Statistical Analysis:

The Fisher exact probability test was used to evaluate significance of enumeration data, such as litters with anomalies.

For quantitative data such as body weights, feed intakes and percent incidence data, the significance was based on multiple comparisons with a nonparametric rank test. ($p < 0.05$, two-tailed).

A test for a linear trend in proportions was used to determine if there was a dose-related change in the proportion of affected litters in groups treated with SC-0224.

Mortality:

Three females, all in the 333 mg/kg/day dose group, died during the study. One female found dead following the 14th dose had a perforated esophagus, indicating death was due to a dosing accident. The other two females were found dead after the second dosing day. Both of these had lungs which were partly dark red, and a darkened (brown or red) cortico-medullary junction in their kidneys. Definite cause of death could not be determined.

Fertility:

Pregnancy percentages were 96, 96, 88, and 88 percent for controls, 30 mg, 100 mg, and 333 mg/kg/day dose groups, respectively. Any differences in fertility between groups were not statistically significant.

Maternal Effects:

Maternal clinical signs which were seen in significantly greater numbers in high dose females (333 mg/kg/day) than in controls or lower doses were chromorhinorrhea, salivation, and lethargy. ($p < 0.05$, two-tailed). However, on necropsy at termination of the study there were no gross findings which could be related to treatment.

In the dams, body weights and feed intake were significantly reduced in the high dose group. The high dose group also showed significantly lower mean uterine weights and liver weights. The relative liver weight for dams receiving 100 mg/kg/day was significantly increased, although the actual mean weight was not affected. A dose-response effect on liver weights was not demonstrated.

Fetal Effects:

There was an increased number of resorptions per dam at the 100 and 333 mg/kg dose levels. (5.7%, 5.6%, 8.6%, and 11.4% for controls, 30 mg, 100 mg, and 333 mg/kg/day dose levels, respectively.) These were mainly early resorptions, and may be related to decreased feed intake in the treated rats. The increases in resorptions are not statistically significant.

There was a statistically significant reduction in mean fetal weight in the 333 mg/kg/day dose group. This, too, could be related to the decreased feed consumption of the dams.

Anomalies:

A number of soft-tissue anomalies were seen in all groups, including controls. These could not be related to treatment or dose. The most numerous ones are shown below: (L = litters; F = fetuses).

	Dose (mg/kg/day)							
	0		33		100		333	
	L	F	L	F	L	F	L	F
	17	65	16	61	19	56	14	49
Brain: dilated 4th ventricle	6	7	3	3	7	9	4	4
Left kidney: dilated pelvis	4	5	4	4	2	2	3	3
Right Kidney: dilated pelvis	17	55	14	43	14	41	9	24
Dilated ureters, left	14	46	13	41	13	37	7	20
Dilated ureters, right	19	76	19	60	14	54	9	27
Convoluted ureters, left	18	61	18	55	14	51	9	26
Convoluted ureters, right								

The most severe soft tissue anomalies were a cleft palate in one fetus in the low dose (33 mg) group and reduced oronasal tissues in one fetus in the high dose (333 mg) group. None of these can be considered a result of treatment.

Regarding skeletal anomalies, those seen in greatest incidence were incompletely ossified sternebrae and lobed vertebral centra; the incidences were similar in controls and all treatment groups.

Five litters in each of the 100 mg and the 333 mg dose groups showed wavy ribs, considered slight but statistically significant ($p < 0.05$, two-tailed). The laboratory quotes Khera in referring to these as fetal aberrations due to delays in normal growth or slight deviations in normal morphogenesis.

No structural teratogenicity was seen in any of the treatment groups.

Conclusions:

1. Maternal toxicity in terms of reduced body weights and feed intake was seen in the high dose group (333 mg/kg/day).

2. Toxic signs were salivation, lethargy after dosing, and increased chromorhinorrhea.

3. Fetotoxicity was seen as reduced fetal body weights at the high dose level. This was apparently a direct result of maternal toxicity.

4. Teratogenicity was not seen in this study.

5. The material tested was stated to be 19.2% pure. Applicant should verify whether doses administered were weights of the 19.2% material or were based on active ingredient.

Data are supplementary.

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Toxicology Branch/HED (TS-769)

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Mutagenicity Evaluation of R-50224 in Sex-Linked Recessive Lethal Test in Drosophila melanogaster.

Litton Bionetics, Inc. Project No. 22169, June 13, 1982

Procedure:

Drosophila Stock Used:

Males of the wild-type stock and females of the Muller-5 stock were used in this study. Newly hatched fruit flies (D. melanogaster) were maintained at 25°C in disposable vials containing the Carolina Biological Instant Drosophila Medium (Formula 4-24, without dyes). Flies were immobilized with filtered CO_2 for handling.

Range-Finding Study:

The toxicity test was performed by the adult feeding methods modified by Lewis and Bacher (1968). A piece of chemically inert glass filter paper lining a shell vial was saturated with 1.5 ml of 1% sucrose solution containing the test compound (0, 12.5, 25.0, and 50 mg/ml). Fifty adult male flies were fed in each vial for 24 hrs. Feeding behavior and toxic effect of the flies were monitored to ensure proper intake of the test compound. The test chemical was found to be non-toxic at the highest concentration tested (50 mg/ml Or 5% W/V).

The fertility results of the treated male flies at each of the selected concentrations are shown in the following table:

Dose	Number Treated	Number Survived	Number Fertile*	Percent Fertility
NC (1% sucrose)	50	48	31	65
12.5 mg/kg	50	47	-	-
25.0 mg/kg	50	48	30	63
50.0 mg/kg	50	43	28	65

* Sufficient numbers of progeny in treated groups were observed for genetic analysis when compared to the control.

Test Size:

The sex-linked recessive lethal test (SLRL) was conducted at two dose levels of the test compound (25 & 50 mg/kg) in conjunction with the negative (1% sucrose) and positive (0.00% M EMS) controls. At least 200 treated male flies were used for each dose level of the test compound. The number of chromosomes tested in the different germ-cell stages were estimated as follows:

	DAYS POST-TREATMENT		
	Brood I	Brood II	Total
	1, 2, 3	4,5,6,7	Chromosomes
NC (1% sucrose)	3750	3750	7500
PC (EMS)	150	150	300
2.5 mg/kg	3750	3750	7500
5.0 mg/kg	3750	3750	7500

P_1 Mating and Rearing of the F_1 Generation:

Following 24-hour waiting period after dosing, the treated males were mated individually to sequential groups of 3 virgin Basc females according to the designed breeding scheme (3-day sequence). The progenies of each male were kept separate and the data can be traced back to individual male used initially. The F_1 progenies of each culture were inspected to make certain the proper cross was made. The desired number of F_1 females was then pair-mated to their brothers. An equal number of F_1 females per treated males was tested to avoid biasing the data.

Scoring of the F_2 Generation:

The F_2 progenies were inspected for the occurance of sex-linked recessive lethals by the presence of males with yellow bodies. If this class of males is absent, the vial was marked as a potential lethal mutation occured on its X chromosome and was set aside for further examination. The following criteria were applied to cultures suspected of being lethal:

(a) If 20 or more progeny were present and there were not yellow-bodied males, the culture was considered to carry a lethal mutation on the treated chromosome and further testing was unnecessary.

(b) If there was less than 20 progeny, or if there was one yellow-bodied male, the culture was retested by mating three of the females heterozygous for the treated and Basc chromosomes to Basc males. The progeny of those crosses were scored for the presence of yellow-bodied males.

Data Analysis:

(a) The total number of X chromosomes tested equaled the sum of lethal and non-lethal cultures. The frequency of X-linked recessive lethal was calculated as:

$$\frac{\text{Number of Lethals}}{\text{No. of Lethals + No. of Non-Lethals}} \times 100 = \% \text{ Lethal}$$

(b) Since the clusters of mutations among progeny from the same treated P_1 males may come from different origins, the cluster was counted as a single event to avoid biasing the interpretation of results.

Interpretation of Results:

A positive mutagenic effect is concluded if the difference in the number of sex-linked recessive lethals between a concurrent control group is statistically significant at the 5% level according to the Kastenbaum-Bowman test (1970). A test is considered negative if the two following criteria are met:

(a) The increase in the treated group over the control is less than 0.2% and the sample size is large enough to allow the detection of a statistically significant increase of 0.2% based on the Kastenbaum-Bowman test.

(b) If none of the broods analyzed shows a positive result, the increases in the treated group of at least two broods must be less than 0.4% and this sample size in each of the two broods must be large enough to allow the detection of a statistically significant increase of 0.4% based on the Kastenbaum-Bowman test.

Results:

Summarized Results in SLRL Test for the Compound R-50224

Dose	No. of F_2 Vials Scored	No. of Lethals	% Lethals
NC (1% sucrose)	Brood I 4033	7	0.17
	Brood II 4235	3	0.07
	Total 8268	10	0.12
25 mg/ml	Brood I 4124	4	0.10
	Brood II 4248	7	0.16
	Total 8372	11	0.13
50 mg/ml	Brood I 4381	5	0.11
	Brood II 4095	6	0.15
	Total 8476	11	0.13
*PC (0.005M EMS)	Brood I 214	101	47.20*
	Brood II 263	98	37.26*
	Total 477	199	41.72*

No significant difference in the percentage of sex-linked recessive lethals was observed in the R-50244 treated groups when compared to the negative control. The increase in the frequency of recessive lethals over the spontaneous frequency for the two broods of treated group (25 or 50 mg/kg) was only 0.01%. Based on the historical data table presented, 5667 flies should be sufficient to detect a significant increase of 0.35% (if the spontaneous background for the tester strain is 0.10%). The sample sizes of the treated groups (8372 to 8476 vials) and the negative control (8268 vials) used in this study were adequate to meet the criteria described in making mutagenicity evaluation of the test compound. The positive control (0.005M EMS) produced an approximately 400-fold increase in recessive lethals over the negative control. Therefore, the test compound, R-50224, was not mutagenic in the SLRL test in Drosophila at the dose levels tested.

Conclusions:

The SLRL test used in this study for detecting the sex-linked lethals in the developing fruit fly between the zygote and the adult stage appears to follow the general guidelines recommended for the sex-linked recessive lethal test in Drosphila melanogaster (EPA 1982). However the following deficiencies in reporting of this study should be clarified:

1. The standard wild-type strain of the male Drosophila (Canton-S or Oregon-R) used in this study was not given and should be identified in the report.

2. The described procedure for scoring of the F_2 generation was incomplete, and should be clarified. The progeny in the F_2 generation should be scored to a fixed end point (generally, not more than 8 days) at 25° C in order to avoid the overlapping of F_3 generation.

3. The study may be up-graded with submission of these clarifications.

Reviewer: John H.S. Chen, DVM
Review Section #1
Toxicology Branch/HED (TS-769)

Mutagenicity Evaluation of SC-0224 in Bone Marrow Cytogenetic Analysis in Rats. Conducted by Jenness B. Majeska, Susan O'Lone, and James Tarca. Stauffer Chemical Co., In Vitro Toxicology Section, Environmental Health Center, Farmington, CT 06032. September 1982. Accession No. 249,802. Report No. T-10884. Caswell No. 893C.

Material Tested:

Trimethylsulfonium carboxymethylaminoethylphosphonate. SC-0224. Lot 6841-48-3. EHC-0293-21. T-10884. Purity 58.5%.

Positive Controls:

Cyclophosphamide in water, given by oral gavage at 30 mg/kg.

Negative and Solvent Controls:

Distilled water.

Animals:

Sprague Dawley adult male rats, Strain CD-CRL: CORS CD (SD)BR, 6 weeks old. Obtained from Charles River Breeding Laboratories.

Dose:

LD_{50} = 565 mg/kg. High dose was 1/3 the LD_{50} (188 mg/kg); intermediate dose was 1/9 the LD_{50} (63 mg/kg); low dose was 1/27 the LD_{50} (21 mg/kg).

Procedure:

In the ACUTE STUDY 128 rats were dosed with single doses of the chemical at 3 levels. In the SUBCHRONIC STUDY 32 rats were dosed with 5 consecutive doses at the same 3 levels. There were 8 POSITIVE CONTROLS. The rats were killed at 6, 12, 27, or 51 hours, or at 5 days after initial dosing.

Three hours before sacrifice the rats were injected intraperitoneally with 4 mg/kg colchicine. They were killed with carbon dioxide inhalation.

The soft tissue and epiphyses of both tibiae were removed and the marrow was aspirated or flushed from the bone, and placed in Hank's balanced salt solution, centrifuged and the pellet resuspended in 0.075 M KCl, centrifuged again and the pellet then resuspended in Carnoy's fixative. After 1/2 hour the fixative was changed and the cells left overnight at 4°C. Slides were prepared of the cells, were air-dried, and then stained with 10% Giemsa at pH 6.8.

If possible 100 spreads were read for each rat, scoring the cells for both structural and numerical aberrations. A mitotic index based on at least 500 cells was recorded.

Data Evaluation and Interpretation:

The testing laboratory describes the evaluation and interpretation of the data as follows:

"Basically, an attempt is made to establish whether a substance or its metabolites can interact with chromosomes to produce gross lesions or changes in chromosome numbers and whether these are of a type which can survive more than one mitotic cycle of the cell. All aberration figures detected by this assay result from breaks in the chromatin which either fail to repair, or repair in atypical combinations. The cell transit time for bone marrow is normally 20 to 24 hours, so an indication of when in the cell cycle a substance is active can often be obtained based on the time of kill.

".... Gaps are not counted as significant aberrations unless they are present in a much higher than usual frequency. Open breaks are considered as indicators of genetic damage, as are configurations resulting from the repair of breaks. The latter includes translocations, multiradials, rings, multicentrics, etc.

".... Cells with more than one aberration are considered to indicate more genetic damage than those containing evidence of single events. Consistent variations from the euploid number are also considered in the evaluation of mutagenic potential."

Results:

TOTAL NUMBERS OF STRUCTURAL ABERRATIONS OBSERVED

Dose	TIME OF SACRIFICE 6 hr.	12 hr.	24 hr.	48 hr.	5 day
Solvent/Vehicle Control	4	2	5	1	1
21 mg/kg	0	0	1	1	0
63 mg/kg	2	1	0	2	1
188 mg/kg	4	1	4	0	0
Positive Control	-	-	24	-	-

As indicated in the above table, SC-0224 did not induce chromosome aberrations in rat bone marrow cells. Compare with the solvent/vehicle controls. The positive controls, however, show a significantly greater number of abberrations ($p < 0.01$, student's t-test). SC-0224 is not mutagenic as determined by this test.

Conclusions:

The assay procedures of this study appears to follow the general guidelines recommended for the In-Vivo Mammalian Bone Marrow Cytogenetic Test (EPA 1982). However, the following inadequacies in reporting of this study must be clarified:

Although mentioned in the report, the described procedures were not adequate to assure the top quality of chromosome spread for aberration analysis.

1. The hypotonic treatment (0.075 M KCl at 37°C) is intended to cause swelling of the cells and spreading of the chromosomes. If the hypotonic treatment is too long or too short, chromosomes will not be spread properly.

2. Cell fixation is normally carried out in three changes of 3:1 methanol: glacial acetic acid, and refrigerated overnight at 4 C. The fixed cells were dispensed on chilled wet slides with subsequent flame warming before being stained in 10% Giemsa.

Mutagenicity Evaluation SC-0224 in Salmonella typhimurium (Lot No. 7269-10). Ames Salmonella/Microsome Mutagenesis Assay. Stauffer Chemical Co. In-Vitro Toxicology Sec. Report No. T-10847. Jan. 19, 1982.

Material Tested:

SC-0224, Lot No. 7269-10. Purity estimated at 90% by weight.

Positive Controls:

Sodium azide, Lot F8A. Eastman Kodak Co.

9-Aminoacridine, purity = 90%. Sigma Chemical Co., Lot. No. 117C-0119.

2-Nitrofluorene, purity = 98%. Aldrich Chemical Co. Lot. No. 112967.

2-Aminoanthracene. Aldrich Chemical Co., Lot. No. 061077.

Indicator Organisms:

Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100 obtained from Dr. Bruce Ames, U. of Cal., Berkeley, Ca.

Metabolic Activation System Reaction mixture: NADP 4mM; Glucose-6-phosphate 5mM; sodium phosphate buffer 100mM; $MgCl_2$ 8mM; KCl 34mM; Homagenate S-9 fraction 100 ul/ml.

Procedure-Plate Test: (Taken from pg. 10 of study report)

Approximately 10^9 cells from an overnight culture of each indicator organism strain growing in nutrient broth were added to separate test tubes containing 2.0 ml molten agar supplemented with biotin and a trace of histidine. In addition, each tube received the designated concentration of test substance (dissolved in water) and either 0.5 ml of phosphate buffer (non-activation) or 0.5 ml of a reaction mixture containing the 9000 Xg liver homogenate supernatant (S-9 activation assay). The dose levels of the test compound in this study were 0.12, 0.37, 1.11, 3.33, and 10 mg/plate in the absence of the S-9 metabolic activation system; and 0.56, 1.11, 1.67, 3.33, 5.0, 10.0, and 15.0 mg/plate in the presence of the S-9 metabolic activation system. The contents of each tube were mixed and then poured onto the surface of selective agar plates and allowed to solidify. The plates were incubated for approximately 48 hours at 37°C and the colonies growing on each plate counted. At least 5 dose levels of the test substance and positive and negative controls were used in each assay.

Preparation of Liver S-9 Supernatant (9000 xg):

Sprague-Dawley male rats or B6C3F$_1$ male mice purchased from the Charles River Breeding Laboratories were used as the source of hepatic/S-9.

500 mg/kg Aroclor 1254 in corn oil was injected I.P. to 200 gram rats or 20 gram mice 5 days prior to sacrifice. They were deprived of food on the evening before sacrifice.

80 mg/kg phenobarbital in corn oil was injected I.P. daily for 5 consecutive days prior to sacrifice.

At sacrifice the livers of 7-10 rats or 20-50 mice were aseptically removed and transferred to sterile ice cold 0.15 M KCl. They were washed with 0.15 M KCl, weighed, minced and diluted with 3 ml of 0.15 M KCl per gram (wet weight) of liver, homogenized with a Potter-Elvehjem or polytron tissue homogenizer.

The homogenate from all animals was pooled prior to being centrifuged for 20 minutes at 9000 xg at 4°C. After centrifugation the S-9 supernatant was transferred by pipetting to storage vials, frozen, and retained at -80°C until used.

Data Evaluation:

Colonies are counted and presented as revertants per plate for each indicator strain. A dose-dependent increase in revertants is considered necessary to establish mutagenicity. If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data lose significance.

Results:

Lot. No. 7269-10. Tested directly without S-9 metabolic activation system. Solvent/Medium control gave averages of 4, 4, 5, 27, and 98/72 revertants per plate for the 5 strains of Salmonella. The test chemical gave similar numbers of revertants at the dose levels tested (0.12 through 10 mg/plate). The positive controls (sodium azide, 9-aminoacridine, and 2-nitrofluorene) gave averages of 444, 69, 300, 295, and 442/438 revertants per plate.

When tested with the S-9 metabolic activation system (rats and mice), no significant differences were observed) between the negative controls and the test chemical (0.56 through 15 mg/plate).

In summary SC-0224 (Lot No. 7269-10) demonstrated no mutagenic activity when tested in the Salmonella/mammalian microsome mutagenicity test.

Conclusion:

The in-vitro Samonella mutagenicity test used for detecting the mutagenic activity of the test compound, SC-0224 (Lot #7269-10) appears to follow the general procedures of Salmonella typhimurium histidine reversion assay described by Ames (Ames et al., 1975). However, the following inadequacies in reporting of this study must be clarified:

1. The described procedures for the preparation of media were unclear and must be clarified:

(a) The source of nutrient broth used in this study must be identified. The variation in the growth of Ames tester strains from the overnight incubation appears to result from variability in the nutrient quality of the medium.

(b) The selective medium should be Vogel Bonner Medium E with 2% glucose. The overlay soft agar should consist of 0.6% purified agar with 0.5 mM L-Histidine-HCl, 0.5 mM biotin, and 0.5% NaCl.

2. The interpretation of results was not clearly given. A test compound which produces neither a statistically significant dose-related increase in the number of revertants nor a statistically significant and reproducible positive response at any one of the test points is considered non-mutagenic.

Mutagenicity Evaluation SC-0224 in Salmonella typhimurium (Lot No. 7646-0901). Ames Salmonella/Microsome Mutagenesis Assay. Stauffer Chemical Co. In-Vitro Toxicology Sec. Report No. T-11070. Sept. 24, 1982.

Material Tested:

SC-0224, Lot No. 7646-0901. Purity: 19.2% by weight.

Positive Controls:

Sodium azide, Lot FBA. Eastman Kodak Co.

9-Aminoacridine, purity = 90%. Sigma Chemical Co., Lot. No. 117C-0119.

2-Nitrofluorene, purity = 98%. Aldrich Chemical Co. Lot. No. 112967.

2-Aminoanthracene. Aldrich Chemical Co., Lot. No. 061077.

Indicator Organisms:

Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100 obtained from Dr. Bruce Ames, U. of Cal., Berkeley, Ca.

Metabolic activation System Reaction mixture: NADP 4mM; Glucose-6-phosphate 5mM; sodium phosphate buffer 100mM; $MgCL_2$ 8mM; KCl 34mM; Homagenate S-9 fraction 100 ul/ml.

Procedure-Plate Test: (Taken from pg. 10 of study report)

Approximately 10^9 cells from an overnight culture of each indicator organism strain growing in nutrient broth were added to separate test tubes containing 2.0 ml molten agar supplemented with biotin and a trace of histidine. In addition, each tube received the designated concentration of test substance and either 0.5 ml of phosphate buffer (non-activation) or 0.5 ml of a reaction mixture containing the 9000 Xg liver homogenate supernatant (S-9 activation assay). The dose levels of the test compound in this study were 0.005, 0.014, 0.041, 0.123, 0.370, 0.617, 1.111, 1.852, 3.333, 5.556, 10.0, 16.667, 25.0, and 50.0 ul/plate in the absence of the S-9 metabolic activation system; and the same concentrations in the presence of the S-9 metabolic activation system using phenobarbital-induced rat liver. Using Arochlor 1254 or phenobarbital-induced mouse liver, concentrations were 0.617, 1.852, 5.556, 16.667 or 50.0 ul/plate. The contents of each tube were mixed and then poured onto the surface of selective agar plates and allowed to solidify. The plates were incubated for approximately 48 hours at 37°C and the colonies growing on each plate counted. At least 5 dose levels of the test substance and positive and negative controls were used in each assay.

Preparation of Liver S-9 Supernatant (9000 xg):

Sprague-Dawley male rats or B6C3F$_1$ male mice purchased from the Charles River Breeding Laboratories were used as the source of hepatic/S-9.

500 mg/kg Araclor 1254 in corn oil was injected I.P. to 200 gram rats or 20 gram mice 5 days prior to sacrifice. They were deprived of food on the evening before sacrifice.

80 mg/kg phenobarbital in corn oil was injected I.P. daily for 5 consecutive days prior to sacrifice.

At sacrifice the livers of 7-10 rats or 20-50 mice were aseptically removed and transferred to sterile ice cold 0.15 M KCl. They were washed with 0.15 M KCl, weighed, minced and diluted with 3 ml of 0.15 M KCl per gram (wet weight) of liver, homogenized with a Potter-Elvehjem or Polytron tissue homogenizer.

The homogenate from all animals was pooled prior to being centrifuged for 20 minutes at 9000 xg at 4°C. After centrifugation the S-9 supernatant was transferred by pipetting to storage vials, frozen, and retained at -80°C until used.

Data Evaluation:

Colonies are counted and presented as revertants per plate for each indicator strain. A dose-dependent increase in revertants is considered necessary to establish mutagenicity. If a chemical produces a response in a single test that connot be reproduced in one or more additional runs, the initial positive test data lose significance.

Results:

Lot No. 7646-0901. Tested directly without S-9 metabolic activation system. Solvent/Medium control gave averages of 13, 5,7, 38, and 163 revertants per plate for the 5 strains of Salmonella. The test chemical gave similar numbers of revertants at the dose levels tested. The positive controls (sodium azide, 9-aminoacridine, and 2-nitrofluorene) gave averages of 988, 144, 872, 662, and 767 revertants per plate.

When tested with the S-9 metabolic activation system (rats and mice), no significant differences were observed between the negative controls and the test chemical.

In summary SC-0224 (Lot No. 7646-0901) demonstrated no mutagenic activity when tested in the Salmonella/mammalian microsome mutagenicity test.

Conclusions:

The in-vitro Samonella mutagenicity test used for detecting the mutagenic activity of the test compound, SC-0224 (Lot #7646-0901) appears to follow the general procedures of Salmonella typhimurium histidine reversion assay described by Ames (Ames et al., 1975). However, the following inadequacies in reporting of this study must be clarified:

1. The described procedures for the preparation of media were unclear and must be clarified:

(a) The source of nutrient broth used in this study must be identified. The variation in the growth of Ames tester strains from the overnight incubation appears to result from variability in the nutrient quality of the medium.

(b) The selective medium should be Vogel Bonner Medium E with 2% glucose. The overlay soft agar should consist of 0.6% purified agar with 0.5 mM L-Histidine-HCl, 0.5 mM biotin, and 0.5% NaCl.

2. The interpretation of results was not clearly given. A test compound which produces neither a statistically significant dose-related increase in the number of revertants nor a statistically significant and reproducible positive response at any one of the test points is considered non-mutagenic.

SC-0224 Mutagenicity Evaluation in the Chinese Hamster Ovary Cytogenetic Assay. Lot #6841-48-3. Stauffer Chemical Co., In-Vitro Toxicology Section Report No. T-10875. July 6, 1982.

Procedure:

Chinese Hamster Ovary Cells were maintained in HAM's F-12 medium (modified by the omission of hypoxanthine) supplemented with 10% fetal calf serum, L-glutamine, penicillin, and streptomycin. Treatment medium is the above growth medium (nonactivation) or serum-free growth medium (activation). The assay is initiated by seeding 8 X 10^5 cells per 75 cm^2 flask in 10 ml of medium. The activation system consists of an S-9 fraction of liver homogenate and necessary cofactors.

In a nonactivation assay cultures are exposed to a test substance from 2-12 hours, colcemid being added to the medium or medium plus chemical 2 to 3 hours prior to fixing the cells. Without S-9 metabolic activation doses of 2, 4, and 6 mg/ml of test substance were used. In an activation assay cultures are exposed to test substances for 2 hours in a serum free medium in the presence of S-9 and cofactors. Cultures then are washed at least twice with phosphate buffer saline and fresh medium added. Colcemid is added 2-3 hours prior to fixing the cells. With S-9 metabolic activation doses of 2, 4, 6, 8, 10, and 12 mg test substance/ml were used.

In the aberration assay cells are treated as above, and 8 to 28 hours after initiating the treatment colcemid is added to the cultures and incubation continued for 2-3 hours.

For the sister chromatid exchange assay, bromodeoxyuridine (final concentration 10uM) is added to the cultures two hours after initiation of treatment. Incubation is continued for another 26 hours, and Colcemid is added 2-3 hours prior to harvest.

Cells are collected by mitotic-shake off, treated with hypotonic KCl, washed twice with methanol:glacial acetic acid fixative, dropped on slides, and air dried. The cells then are stained and are scored for aberrations, and 20 metaphases are scored for sister chromatid exchanges.

Results:

Aberrations:

In the non activation portion of the assay increased aberrations over controls were seen at 4 mg/ml ($p<0.05$).

In the activation portion, increased chromosomal aberrations were seen at 8, 10, and 12 mg/ml. $p<0.01$ at 10 and 12 mg/ml.

Sister chromatid exchange was not determined.

Conclusions:

The in-vitro cytogenetic assay in CHO cells used for this study appears to follow the general guidelines recommended for the in-vitro mammalian cytogenetics and in-vitro sister chromatid exchange assay (EPA 1982, OECD 1981). However, the following inadequacies in reporting of this study must be clarified:

1. Although mentioned in the report, the described procedures were not adequate to assure the top quality of chromosome spread for aberration or sister chromatid exchange analysis.

(a) The hypotonic treatment (0.075 M KCl at 37 C) is intended to cause swelling of the cells and spreading of the chromosomes. If the hypotonic treatment is too long or too short, chromosomes will not be spread properly.

(b) Cell fixation is normally carried out in three changes of 3:1 methanol:glacial acetic acid, and refrigerated overnight at 4 C. The fixed cells were dispensed on chilled wet slides with subsequent flame warming before being stained in 10% Giemsa.

2. In the Non-activation Assay treatment, it is stated that "Cultures are exposed to a test substance from 2 - 12 hours", and that the exposure time will be indicated on the raw data. Since the raw data were not provided, the exposure time should be included in the report.

SC-0224 Mutagenicity Evaluation in the Chinese Hamster Ovary Cytogenetic Assay. Lot #7466-18-01. Stauffer Chemical Co., In-Vitro Toxicology Section Report No. T-11019. July 22, 1982.

Procedure:

Chinese Hamster Ovary Cells were maintained in HAM's F-12 medium (modified by the omission of hypoxanthine) supplemented with 10% fetal calf serum, L-glutamine, penicillin, and streptomycin. Treatment medium is the above growth medium (nonactivation) or serum-free growth medium (activation). The assay is initiated by seeding 8 X 10^5 cells per 75 cm^2 flask in 10 ml of medium. The activation system consists of an S-9 fraction of liver homogenate and necessary cofactors.

In a nonactivation assay cultures are exposed to a test substance from 2-12 hours, colcemid being added to the medium or medium plus chemical 2 to 3 hours prior to fixing the cells. In an activation assay cultures are exposed to test substances for 2 hours in a serum free medium in the presence of S-9 and cofactors. Cultures then are washed at least twice with phosphate buffer saline and fresh medium added. Colcemid is added 2-3 hours prior to fixing the cells. With S-9 metabolic activation doses of 1, 2, 3, 4, 5, 6, 7, and 8 ul test substance/ml were used.

In the aberration assay cells are treated as above, and 8 to 28 hours after initiating the treatment colcemid is added to the cultures and incubation continued for 2-3 hours.

For the sister chromatid exchange assay, bromodeoxyuridine (final concentration 10uM) is added to the cultures two hours after initiation of treatment. Incubation is continued for another 26 hours, and colcemid is added 2-3 hours prior to harverst.

Cells are collected by mitotic shake-off, treated with hypotonic KCl, washed twice with methanol:glacial acetic acid fixative, dropped on slides, and air dried. The cells then are stained and are scored for aberrations, and 20 metaphases are scored for sister chromatid exchanges.

Results:

Aberrations:

In the activation assay, increased chromosomal aberrations were seen at 6, 7, and 8 ul/ml of the test substance ($p < 0.01$).

Sister Chromatid Exchange: No significant increase in sister chromatid exchanges at the dose levels tested (1 through 8 ul/ml) in the presence of S-9 metabolic activation system.

Conclusions:

The in-vitro cytogenetic assay in CHO cells used for this study appears to follow the general guidelines recommended for the in-vitro mammalian cytogenetics and in-vitro sister chromatid exchange assay (EPA 1982, OECD 1981). However, the following inadequacies in reporting of this study must be clarified:

1. SC-0224 induces chromosomal aberrations in Chinese hamster ovary cells in the presence of Aroclor 1254 induced rat liver metabolic activation.

2. Although mentioned in the report, the described procedures were not adequate to assure the top quality of chromosome spread for aberration or sister chromatid exchange analysis.

(a) The hypotonic treatment (0.075 M KCl at 37°C) is intended to cause swelling of the cells and spreading of the chromosomes. If the hypotonic treatment is too long or too short, chromosomes will not be spread properly.

(b) Cell fixation is normally carried out in three changes of 3:1 methanol:glacial acetic acid, and refrigerated overnight at 4°C. The fixed cells were dispensed on chilled wet slides with subsequent flame warming before being stained in 10% Giemsa.

3. In the Non-activation Assay treatment, it is stated that "Cultures are exposed to a test substance from 2 - 12 hours," and that the exposure time will be indicated on the raw data. Since the raw data were not provided, the exposure time should be included in the report.

SC-0224 Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test-Forward Mutation Assay. Study T-10844. Lot. Nos. 7269-10 and 6841-18-3. By Stauffer Chemical Company. In-Vitro Toxicology Section; Farmington, Ct. Feb. 8, 1982.

Procedure:

Indicator cells are from the L5178Y ($TK^{+/-}$) cell line from the 3.7.2 clone of Fischer L 5178Y cells furnished by Dr. Donald Clive, and are maintained until needed in frozen nitrogen.

For metabolic activation to the treatment medium are added S-9 homogenate, 240 ug NADP, and 450 ug isocitrate.

The test compound was tested for mutagenic effects on L5178Y ($TK^{+/-}$) mouse lymphoma cells in vitro. The investigation were performed with microsomal activities at concentrations of 0.375, 0.750, 1.50, 3.00, 6.00, 8.0, 8.5, 9.0, 9.5, and 10 mg/ml and without microsomal activation at concentrations of 0.375, 0.75, 1.50 3.0, 6.0, 7.0, 8.0, 9.0 and 10 mg/ml. The mutagenicity test was carried out by treating L5178Y ($TK^{+/-}$) cells with the selected concentrations at a cell density of 12 X 10^6 cells for 4 hours. Following exposure the substance is removed and the cells are resuspended in 40 ml of media. A portion of the cell suspension is used for the forward mutation assay described by Clive (Clive, et al. 1975 and 1979).

Evaluation:

Mutation Assay:

To be acceptable, the cloning efficiency of solvent/vehicle controls should be 75% or greater. Values greater than 100% are possible because of errors in cell counts and dilutions in cloning. Cloning efficiencies in the range of 50% to 70% may be conditionally acceptable. Generally in order to call a susbtance non-mutagenic it should be tested within a dose range which induces 80% to 90% toxicity at the higher limits unless prevented by insolubility.

$TK^{+/-}$ Mouse Lymphoma Mutation Assay: A substance is considered mutagenic if it produces a dose-dependent increase in mutation frequency over 3 doses to a level at least 2.5 times the solvent/vehicle control.

Results:

1. Toxicity was seen at doses exceeding 7 mg/ml.

2. In the direct assay, insufficient effect was seen to indicate a positive result.

3. With metabolic activation there was a significant reproducible increase in mutation frequency. This indicates mutagenicity at the thymidine kinase locus in L5178Y mouse lymphoma cells under S-9 rat liver metabolic activation.

Conclusions:

The assay used to evaluate the mutagenic activity of the test compound, SC-0224 (Lot #7269-10 and 6841-48-3) in the L5178Y/TK$^{+/-}$ mouse lymphoma cells with and without S9 metabolic activation system, appears to follow the procedures described by Clive (1975 and 1979). However, the data presented in Table 4 were confusing and must be corrected. These data presume to be the results of repeated study under activation test conditions instead of the results from non-activation assay.

SC-0224 Morphological Transformation of BALB/3T Cells. (Lot #729-10). Report T-10849. Stauffer Chemical Co., In Vitro Toxicology Section, Farmington, Ct. January 4, 1982.

Indicator Cells Used:

1-1 subclone of clone A-31 of BALB/3T3 mouse cells obtained from Dr. Takeo Kakunaga of NCI, or the 8 below clone C-14 of clone CI-13.

Medium:

Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum or new born calf serum, glutamine (2mM), penicillin (100 units ml), and streptomycin (100 ug/ml).

Dose Selection:

Dishes are seeded 24 hours before dosing with 200 cells per dish. After 3 days exposure to the test substance the cells are washed and incubated in growth medium for an additional 6-10 days. When the colonies reach adequate size (3 to 7 mm), the plates are fixed, stained, and counted. Relative survival of each dose = $\frac{\text{no. of colonies surviving treatment}}{\text{no. of colonies in solvent/vehicle control dishes.}}$

Transformation Assay Procedure: Described by Kakunaga (1973).

Cells are plated at density of 1 X 10^4 cells/T-25 flask 24 hours prior to treatment. Fifteen flasks are treated for each of the 5 doses of the test chemical (0.313, 0.625, 1.25, 2.50, and 5.0 mg/ml), a positive control, and two negative (solvent/medium and medium) controls. After 3 days exposure, the cells are washed and refed. Flasks are refed twice weekly for 4-6 weeks. The assay is then fixed unless transformed foci have not been observed in positive control flasks. A concurrent toxicity assay for the solvent/vehicle control and treatment doses may be included with each transformation.

The results were presented as the number of foci per set of cultures for each dose level. The medium/solvent control and positive control, 3-methylcholanthrene, were run concurrently.

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Results:

1. 3 mg/ml was the lowest toxic dose.

2. There was no increased number of transformed foci over controls in the treated Balb/3T3 cell culture when compared to the negative control.

Conclusions:

The assay which evaluates the test compound for its ability to induce malignant transformation of Balb/3T3 cells in vitro appears to follow the general procedures described by Kakunaga (Int. J. Cancer 12:463, 1973). However, the following inadequacies in reporting of this study must be clarified:

Two different culture vessels (dishes or T-25 flask) were mentioned in this report. If the dishes were employed in this study, CO_2 concentration, temperature and relative humidity are critical factors in maintaining the proper cell growth. The specific incubator for propagating the cell cultures should be identified.

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Acute Oral Toxicity of SC-0224 4-LC Formulation in Male and Female Sprague-Dawley Rats. Conducted by S. Sorenson, B.J. Jones, D. Crume, and T.R. Billow. Stauffer Chemical Co. Richmond Toxicology Laboratory, Richmond, California. Report T-11189. November, 1982. Accession No. 249803, Caswell No. 893 C.

Material Tested:

SC-0224 4LC. Lot # 8014-42. Formulation contains 41.4% w/w trimethylsulfonium carboxymethylaminomethyl phosphonate, [redacted]

Procedure:

Male Rats:

After 16-18 hours fasting ten male rats per dose level were treated by oral gavage at the rate of 650, 800, 900, and 1000 mg/kg formulation suspended in corn oil. Twenty rats were dosed with water for negative controls. Mortality was 8/10, 6/10, 4/10, and 0/10 for doses of 1000 mg/kg, 900 mg/kg, 800 mg/kg, and 650 mg/kg, respectively. The oral LD_{50} for male rats was 846 mg/kg, with 95% confidence limits of 762-940 mg/kg.

Female Rats:

After 16-18 hours fasting, ten female rats per dose level were treated by oral gavage at the rate of 1000, 900, 800, 700, and 650 mg/kg formulation suspended in corn oil. Twenty rats were dosed with water for negative controls. Mortality was 8/10, 7/10, 7/10, 0/10, and 1/10 for doses of 1000 mg/kg, 900 mg/kg, 800 mg/kg, 700 mg/kg, and 650 mg/kg, respectively. The oral LD_{50} for female rats was 805 mg/kg, with 95% confidence limits of 726-892 mg/kg.

Clinical Signs:

All mortalities occurred within 24 hours. Clinical Signs were depression, hypopnea, ptosis, piloerection; ataxia, and occasionally tremors and prostration. Survivors appeared normal by days 5 or 6.

Necropsy findings in rats dying on test were darkened lungs, livers, and spleens and clear fluid in the GI tracts of rats on the high dose levels. There were no visible lesions in rats on the lower dose levels or in those surviving to 14 days.

Twenty males and 20 females dosed with water appeared normal throughout the 14-day observation period and on necropsy at day 14.

The data meet Core-Minimum Standards.

Toxicity Category III.

Acute Dermal Toxicity of SC-0224 4-LC Formulation in Stauffland Albino Rabbits - Abraded Skin. Conducted by T. Billow, et al.; Stauffer Chemical Co.; Richmond Toxicology Laboratory; Richmond, California. Report T-11189. November, 1982. Accession No. 249803. Caswell No. 893 C.

Material Tested:

SC-0224 4LC. Lot #8014-42. Formulation contains 41.4% w/w trimethylsulfonium carboxymethylaminomethyl phosphonate, [redacted]

Procedure:

Four male and female Stauffland albino rabbits per dose level had the test material applied to the clipped abraded abdominal skin under a protective cover. Dose levels were 1200, 1000, 800, 650, 550, and 450 mg/kg body weight. Three male and 3 female rabbits served as zero dose controls. After 24 hours the binder and the test material were removed, the treatment sites were inspected for irritation, and the abdomens were rewrapped in a gauze binder. Three days later the gauze binder was removed and the rabbits were observed for 14 days following initial treatment. Necropsies were performed on all rabbits that died during the study and on all survivors at 14 days.

Results:

Four of 8 rabbits died at 1200 mg/kg; 4 of 8 at 1000 mg/kg; 3 of 8 at 800 mg/kg; 2 of 8 at 650 mg/kg; and 1 of 8 at 550 mg/kg. There was no mortality at 450 mg/kg or in the controls.

Necropsy Effects seen in rabbits that died during the study included pale lungs in 2 rabbits at 1000 mg/kg; a red and purple area between the right kidney fat and abdominal wall in 1 rabbit at 1000 mg/kg; darkened lungs in 1 rabbit at 800 mg/kg; and reddened saliva in 1 rabbit at 800 mg/kg. A red and purple area also was seen on the inside wall of the abdominal cavity in 1 rabbit on 550 mg/kg. No other findings were seen on necropsy.

Clinical Signs were mild to severe depression in some rabbits at all treatment levels. Salivation, prostration, and tremors also were seen in some rabbits, with no particular regard to dose level.

Local Dermal Effects was a mild to moderate erythema and mild to moderate edema after 24 hours exposure, but without any relation to dose level.

No clinical, necropsy, or dermal effects were seen in the controls.

LD_{50} (abraded skin) = 1061 mg/kg - male and female rabbits combined. 95% confidence limits = 759 - 1483 mg/kg.

Acute Dermal Toxicity of SC-0224 4LC Formulation in Stauffland Albino Rabbits - Intact Skin.

This portion of the study was conducted in the same manner as for the abraded skin. Doses of 2000, 1850, 1700, 1500, 1200, 1000, and 800 mg/kg were used. Mortality was 8/8 at 2000 mg/kg; 6/8 at 1850 mg/kg; 4/8 at 1700, 1500, and 1200 mg/kg; 2/8 at 1000 and 800 mg/kg.

The acute dermal LD_{50} in intact skin was 1316 mg/kg, with 95% confidence limits of 1007-1722 mg/kg.

Clinical signs and necropsy findings in rabbits treated on intact skin were similar to those with abraded skin.

Combined LD_{50}, intact and abraded skin = 1279 mg/kg, 95% confidence limits for LD_{50} = 999-1639 mg/kg. Differences between intact skin and abraded skin rabbits were not statistically significant at $p > 0.05$ (by method of Litchfield and Wilcoxon).

Conclusions:

1. Acute dermal LD_{50} in male and female rabbits (intact and abraded skin) = 1279 mg/kg. 95% confidence limits for LD_{50} = 999-1639 mg/kg. Dermally, the formulation is slightly more acutely toxic than the technical chemical.

2. Toxicity Category II.

3. Study meets Core Minimum Requirements.

Primary Skin Irritation of SC-0224 - 4LC Formulation in Stauffland Albino Rabbits. Conducted by T.R. Billow and D. Crume. Stauffer Chemical Co. Richmond Toxicology Laboratory; Richmond, California. Report T-11189. November 1982. Accession No. 249,803. Caswell No. 893C.

Material Tested:

SC-0224 4LC. Lot#8014-42, Formulation contains 41.4% w/w trimethylsulfonium carboxymethylamino methylphosphonate;

[redacted]

Procedure:

Twenty-four Hour Exposure:

Six Stauffland albino rabbits were used in the study. One-half ml of SC-0224 4LC formulation was placed on an abraded site and an intact site and covered with a one-inch square gauze patch. This was secured by adhesive tape and wrapped with rubberized damming for 24 hours. After 24 hours the coverings and test material were removed and the reactions scored. The site reactions were also scored after 72 hours.

Results of 24-Hour Exposure:

At 24 hours mild to moderate erythema and edema were seen in the intact and abraded skin of the 6 rabbits. At 72 hours mild edema and scar tissue were seen in all rabbits. The Primary Irritation Score (Draize) was 2.92.

Four-Hour Exposure:

The previous procedure was also conducted with an exposure time of 4 hours and observations and scoring being made after 4 hours, 24 hours, and 72 hours.

After 4 hours mild to moderate erythema in 5 rabbits and cracked skin in 1 rabbit were seen in the intact and abraded skin. At 72 hours, mild erythema was seen in 4 rabbits and moderate erythema in 1 rabbit. One rabbit was found dead prior to the 4-hour reading. The primary irritation score was 1.27.

[redacted]

Conclusions:

1. SC-0224 4LC Formulation is a moderate dermal irritant.

2. Toxicity Category III.

3. Data are Core Minimum.

Primary Eye Irritation of SC-0224 4LC Formulation in Stauffland Albino Rabbits. Conducted by T.R. Billow and D. Cruma. Stauffer Chemical Company. Richmond Toxicology Laboratory. Richmond, California. Report T-11189. November, 1982. Accession No. 249,803. Caswell No. 893C.

Material Tested:

SC-0224 4LC. Lot #8014-42. Formulation contains 41.4% w/w trimethylsulfonium carboxymethylaminomethylphosphonate; [redacted]

Procedure:

Nine rabbits were treated by placing 0.1 ml of the formulation inside the lower lid of one eye of each rabbit. In 3 of the rabbits the eye was washed with water 20-30 seconds after exposure, and in the remaining 6 rabbits the eye was not washed. Untreated eyes served as negative controls. The cornea, iris, and conjunctivae were observed at 24, 48, and 72 hours, and at 4, 7, 10, 14, 16, 17, 21, and 24 days after treatment. Scoring was according to the method of Draize.

Results:

In the six rabbits whose eyes were not washed mild to severe corneal opacity, moderate iritis, and moderate to severe conjunctival irritation were seen. This persisted through 10 and 14 days, with moderate to severe corneal opacity and mild conjunctivitis still observed in 2 rabbits at 24 days.

In the three rabbits whose eyes were washed, mild corneal opacity and mild iritis were produced in 2 rabbits, with mild to severe conjunctivitis seen in all 3 rabbits. By day 24 the only observation in these rabbits was mild conjunctivitis in 2 rabbits.

[redacted]

Conclusions:

1. SC-0224 4LC formulation is very corrosive to the eye, producing corneal opacity, iritis, and severe conjunctivitis. This is in contrast to the technical chemical which is only mildly irritating to the eye.

2. The irritation and corrosiveness is reduced somewhat by washing the eyes.

3. Toxicity Category I.

4. The data are Core Minimum.

TS-769:GESSERT:sll:X73710:10/27/83 card 5

DCR-44903:Gessert:12/13/83:HED-05:del. 12/29/83:LM
REVISED-12/7/83:pac was 44971